DETECTION OF NASALBOT FLY LARVAE IN SLAUGHTERED SHEEP HEADS IN ALSALAM ABATTOIR IN KHARTOUM STATE –SUDAN

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بسم الله الرحمن الرحيم

قال تعالى : وانّ لكم في الانعام لعبرة نسقيكم مما في بطونه من بين فرث ودم لبناً خالصاً سائقاً للشاربين .

صدق الله العظيم

سورة النحل

الأية (66)

DEDICATION

To the souls of my mother and father.

To my husband, my sons and daughters.

My sister and brothers.

To who wish and work for peace in Darfur

With great love and gratitude.

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ABSTRACT

A study was carried out to detect nasal botfly larvae in slaughtered sheep heads during March and May 2009 in Alsalam Abattoir, Ombedda locality, Khartoum State, Sudan. A total of 567 heads of sheep were collected using convenience sampling technique, opened and examined for *Oestrus ovis* larvae infestations. This study show nasal myiasis among the examined sheep heads with prevalence rate of 17.4%.

A total of 151 larvae were collected and identified using Zumpt key with the aid of a dissection microscope .The maximum numbers of larvae collected from only one sheep head was 13 larvae of different stages, 1.5 larvae is the mean larval burden. One hundred thirty six larvae were third instars (90.1%), 15 larvae were second instars (9.9 %) and no first instars were found.

This study show that the incubation period and transformation from pupa to adult fly were differ with different season of year and different degrees of temperature, so after incubation of the collected larvae at 38^{o}C, the pupation period was two to three weeks with 65.6% pupation. The pupation period ranged between 17 and 23 days with 57.4% pupation when the larvae were incubated at 28^{o}C .All emerged flies were similar in morphology and belonged to *Oestrus ovis*. The emerging flies remained viable for more than eleven days in covered Petri dishes before they started to die.

It is concluded that sheep nasal myiasis is wide spread in Sudan and the only causal agent is the larvae of *Oestrus ovis*.

Due to the importance of such infestation, it is concluded that more investigation for assessment of its economic losses to sheep is needed as a prerequisite for control.

الكشف عن يرقات ذبابة نغف أنف الأغنام في رؤوس الضأن التي ذبحت في مسلخ السلام بمحلية أمبدة, ولاية الخرطوم ـ السودان

المستخلص:-

أجريت هذه الدراسة لتحديد مدي انتشار حالات تدويد الأنف في الضأن بالسودان وذلك بالبحث عن يرقات ذبابة نغف أنف الأغنام في رؤوس الضأن التي ذبحت في مسلخ السلام بمحلية أمبدة، ولاية الخرطوم بالسودان خلال شهري مارس ومايو 2009م. وقد جمعت 151 يرقة وتم التعرف عليها باستخدام مفتاح عالم الطفيلياتZumpt ومساعدة مجهر التشريح.

أكبر عدد من اليرقات تم جمعها من رأس واحد من الضأن هو13 يرقة بأطوار مختلفة بمتوسط حمل1.5 يرقة بكل رأس تم فتحه. كانت يرقة 136 (90.1%) من الطور الثاني و15 (9.9) من الطور الثالث ولم يتبين يرقات الطور الأول.

أظهرت الدراسة أن فترة الحضانة والتحول من شرنقة إلي حشرة كاملة تختلف باختلاف فصول السنة واختلاف درجة الحرارة،حيث انه عند وضع اليرقات التي جمعت تحت درجة الحرارة 38درجة مئوية امتدت فترة الحضانة لفترة تتراوح مابين أسبوعين إلي ثلاثة أسابيع ونسبة تحول 65.6% فيما بلغت نسبة التحول 54.4% تحت درجة حرارة 28درجة مئوية وفترة حضانة امتدت مابين 17و23يوم.

أظهرت الدراسة أن جميع الذباب متشابه في شكله الخارجي وأنها تنتمي إلي جنس ذبابة نغف أنف الأغنام وبقي الذباب حياً لفترة تزيد عن احدي عشر يوما عند حفظها في أطباق بتري قبل أن تبدأ في الموت.

خلصت الدراسة إلي أن حالات تدويد الأنف في الأغنام منتشرة في السودان وأن السبب الوحيد له هو يرقات ذبابة نغف أنف الأغنام (*Oestrus ovis*).

نظراً لأهمية حالات تدويد الأنف في الأغنام فأن الحاجة ماسة لتقيم التأثير الاقتصادي قبل البدء في المكافحة.

CHAPTER ONE

INTRODUCTION

Sudan is at the top of the Arab and African countries in animal population density, animal products and productivity .The estimation of animal population in the Sudan is about (136 mil) heads, the estimation of sheep is about (49.8 mil) which constitute (36.6%) of all animal population according to the records of the Ministry of Animal Resources and Fisheries (2005).http://www.sudan.gov.sd /en/index.ph option (2008).

Sheep are distributed all over the Sudan with different densities in different states depending on the climate and type of grazing. The highest density of sheep population is found in Kordofan, Darfur, White Nile and Blue Nile States .Sheep play an important role in the economic cycle in the rural and urban areas as a source of meat, leather and wool. Sheep and sheep products form the largest part in the Sudan export.

Myiases are animal and human diseases caused by parasitic dipterous fly larvae feeding on the host necrotic and living tissues, liquid body substances or ingested food.

Sheep nasal botfly is the member of the order Diptera. Its larvae are well known as myiasis producing agents .The fly is small grey green with black spots on the thorax, and covered with short brown hairs .It is widely spread in the tropics throughout the year specially in the hot season.

A female fly deposits eggs or young larvae around the nostrils of sheep or goats. When the eggs hatch the larvae, armed with two hooks on the anterior end crawl up the nostrils feeding on the nasal mucosa and enter the sinuses where they grow. After 9-10 months they pass through second stage of development and migrate to become mature in the frontal sinuses and finally they are sneezed out. Some larvae may die after development when the entrance becomes small and they become unable to get out. The larvae, yellow white in colour, drop to the

ground. They then barrow into the soil. Pupation takes place during 3-6 weeks after which the young fly emerges (Hall.1977).

Infestation of sheep with the larvae of the nasal botfly, *Oestrus ovis*, has-been recognized since early times, the first case was recorded in Australia in 1906.Although there is considerable controversy about its pathogenic significance (some regard it as benign) but many others hold that the wellbeing of infested animal may profoundly be affected (Du Toit and Fiedler, 1956), cited by Zumpt(1965).

Infestation of sheep with the nasal botfly indirectly affects sheep health and sheep production by directly affecting the weight gain, milk yield and fleece production. Annoyance and irritation by *Oestrus ovis* larvae causes sheep to lose valuable grazing time, and suffer from mucopurulent discharge and difficult respiration. It also causes pathological damage of the nasal cavities.

Oestrus ovis infestation occasionally occurs in humans associated with ocular myiasis, oral myiasis and stomatitis.

Although sheep suffer from massive ill health due to *Oestrus ovis* infestation the condition is generally regarded as relatively benign and no attention is directed to it and there is no information available about the incidence of sheep botflies in Sudan and no attention is given for its control.

Alsalam abattoir receives sheep from ELmowelih livestock market in Omdurman which is supplied with sheep from different states of Sudan , specially Western and Central Sudan , which are the sites of high sheep production .

This study is conducted to achieve the following objectives:

1. To determine the infestation rate with nasal botflies in sheep slaughtered in Alsalam abattoir (during March and May 2009.)
2. To determine the intensity of larval infestation in sheep heads
3. To identify the flies that emerge from the larvae collected from the dissected sheep heads.

CHAPTER TWO

LITERATURE REVIEW

2.1 Order Diptera:

The Diptera are some of the largest orders of insects including over 6400 species. Structurally they are highly specialized members of their class. Almost all the species are diurnal and the majority are either flower–loving which feed upon nectar or of decaying organic matter of various kinds, but a numbers of the flies are predacious and live on various insects .Some diptera have acquired blood–suckling habits ,attacking man and other vertebrates excepting fishes .The greatest number of segments in their larva is twelve e.g. Three thoracic and the nine abdominal (Richards and Davies 1960) .

2.1.1. Oestridae:

Oestridae are more encountered as larvae than as adults, and many species are described from their larval stage only. The larvae are broadly cylindrical or barrel –shaped, narrowing at their extremities when they are mature but never taper anteriorly. The body -wall is very tough with lateral swelling and a group of spinules in twelve segments are present. The first two are much reduced and annular.

As a rule Oestrid larvae are white in colour and opaque, the anterior spiracle lying in a deep pit. (Richards and Davies 1960).

The larvae feed upon serous and other exudations from the tissues of their hosts. These fluids are usually either altered or increased due to irritation induced by the presence of the parasite .When mature, the larvae leave their hosts and pupate in the ground or among surface litter (Richards and Davies 1960).

The sheep nasal fly (*Oestrus ovis*) is usually larviparous depositing its larvae in the nostrils of sheep. When the larvae mature they release their hold and leave the animal. The presence of larvae in the nostrils causes nasal discharge in sheep and often obstruction of its air passages.

2.2. Myiasis:

Myiasis is an animal or human disease caused by parasitic dipterous fly larvae feeding on the host necrotic or living tissue, generally termed "fly strike" and "fly blow".

German Entomologist Zumpt (1965) described myiasis as the infection of live human and vertebrate animals with dipterous larvae which feed on the host's living or dead tissue, liquid, body substance or ingested food.

2.2.1. The Life cycle of myiasis causing larvae other than nasal bot flies:-

Blowfly strike known as myiasis is a common disease of sheep specially in an area where there are hot and wet conditions, the female flies lay their eggs on the sheep at areas soiled with urine and faeces mainly on the buttocks. The eggs take 8-24 hours to hatch depending on the right conditions , the larvae lacerate the skin resulting in sores and early removal of lamb's tails. The larvae then move into the host's tissues causing irritating lesions which are invaded by bacteria leading to toxemia or septicemia, anorexia and weakness. If untreated this will lead to death. Blowfly strike account for over $ 170 million a year in losses in the Australian sheep industry. Prevention measures such as mulesing is practiced (Belschner *et al* 1956).

2.2.2. Classification:

There are two different classifications of myiasis.The classical one describes myiasis by the infected part of the host i.e. dermal, sub dermal, cutaneous, nasopharyngeal, ocular, intestinal/enteric or urogenetal. The other classification is based on the relationship between the host and the parasite depending on the biology of the flies' species causing myiasis and it is pathogenecity, thus it is described as obligatory, facultative or accidental.

2.2.3. Flies responsible for myiasis:

Three main fly families are responsible for causing economically important myiasis in livestock and humans.

1. Oestroidae(botflies)
2. Calliphoridae (flesh flies).
3. Sarcophagiadae (flesh flies).

But other families such as Anisopodidae, Piophilidae, stratiomyidae and syrphidae are occasionally involved, Wikipedia org.(2008).Other important species mainly causing human myiasis are *Dermatobia* hominis (human botfly) and *Cordylobia anthropophaga*(tumbu fly) Hakimi and Yazdi (2002).

The adult flies are not parasitic but their larval stages (known as maggots or grubs) feed on live or necrotic tissue causing myiasis to develop. They may be ingested or may enter through any body apertures.

Alahmed, 2004 reported the presence of *Chrysomya bezziana*, *Chrysomya albiceps and Wohlfahtia* nuba from Sudi Arabia.

2.2.4. Control Methods:

1. Vector control: This aims at the control of adult flies before they can cause any damages i.e. prevention.

2. Treatment**:** This is given once the infection has taken place.

2.2.4.1. Prevention.

Application of insecticide in the environment, where the target Livestock is kept, using organophosphorus or organochlorine compounds in spray formulation.

SIT (Sterile Insect Technique). A significant number of artificially reared sterilized male flies are introduced to compete with wild bred males for females in order to copulate and cause the female to lay batches of un fertilized eggs which can not develop into larval stages. Removing the environment most

favorable to flies i.e. crutching of sheep (Removal of wool from-around the tail and between the legs of sheep).

The study of the efficacy of two pour-on formulations containing cyromazine for the prevention of cutaneous myiasis of sheep are presented by Lonsdale *et al* (1990).He found that the incidence of cutaneous myiasis is reduced by 87 %-100% for 8 weeks when formulation of 6%w/v cyromazine was used at an application rate of 60—85mg of active ingredient /kg body weight. Sheep treated with 10% w/v cyromazine at 50 or 100 mg/kg and exposed to adult flies are found to be completely protected for nine and eight weeks respectively, (Lonsdale *et al* 1990).

2.2.4.2. Treatment: -

This is applied when the animal is infested, such as using of slow released boluses of Ivermectin to provide long term protection against larva, development, Spraying and drenching, whole body dipping is better than spraying.(Sainsbury,1998)

2.2.5. Use of Myiastic Maggots in Medicine:

Maggots have been used in medicine in order to clean out necrotic wounds. Maggots therapy are also known as Maggot Debridement Therapy (M.D.T) or larval therapy e.g. Green bottle fly larvae are used exclusively for this purpose. (Wikipedia org/wiki/myiasis 2008).

2.3. *OESTRUS OVIS*

2.3.1. Classification:

Phylum: Arthropoda.

Class: Insect.

Subclass: Apterygota.

Order: Diptera.

Sub Order: Cyclorrhapha.

Family: Oestridae.

Sub family: Oestrinae

Genus: Oestrus.

Species: *Oestrus ovis.*

Similar Oestrus Flies are:-

1- Genus Rhinoestrus: The members of this Genus parasitized horses, donkeys, mules and Zebra.

2- Genus Gedoelstia: The members of this Genus parasitized black and blue wild beest.

3-Genus Cephalopina Strand: This Genus contains one species, which parasitized domestic camels.Zumpt (1965) .

Genus Oestrus include five species – four are parasites of antelopes and , *Oestrus ovis* parasitizes sheep and goats (Howard.1980) ,cited by Kettle (2000).

A comprehensive list of the literature on *Oestrus .ovis* in the period (1686-1973) is given by Papavero (1977),cited by Kettle (2000) .*Oestrus ovis* achieving world-wide distribution by being spreaded throughout the world via sheep exportation. (Kettle *et al*2000).

2.3.2. History:

The nasal bot of sheep was described by Zumpt (1965) , also it was described by other pre-Linnaean authors ,(Zumpt (1965) .

The first comprehensive paper on the bionomics, control measures of myiasis and its relation to man was written by Portschinsky (1936), cited by Zump(1965).This was followed by a number of shorter papers by various authors who dealt with it where in its taxonomy, biology, veterinary and economic importance were given. Sergent (1952) wrote a basic paper on human ophthalmomyiasis, cited by Zumpt (1965).

In Libya Gabaj, *et al* (1992) reported *Oestrus ovis* prevalence rate of 22.6 and 18.4% in sheep and goat respectively.

2.3.3. Morphology:

2.3.3.1. Adult flies:

The adult fly has primitive none functional mouth parts (Urquhart *et al* 1996).The adult flies are quite well characterized and it is very easy to identify them. The adult fly has a yellow-brown head with dense glossy bottomed black pits dorsally between the eyes on the parafrontalia , and black tubercles among the yellow hairs on the yellow-brown scutum and scutellum . The abdomen is black with an irregular pattern of lighter marking varying with the angle of illumination. In the male the frons is at its narrowest point a little less than the half eye length . In the female it is broader than one eye in length. Parafacialia with a pale hair situated in a pale or slight darkened foot-characteristic feature for separating *Oestrus ovis* from *Oestrus aureoargentatus*. The wings have yellow veins, the legs are yellow or yellow-brown in colour, the abdomen is completely black or reddish – brown with a speckled grayish or white colour according to the light incidence .

The body is about 10 -12 mm in length Zumpt, (1965).The adult sheep botfly does not feed. (Campbell *et al*1985), Plate (1).

2.3.3.2. First stage larvae:-

They are spindle in shape 1-3mm long with relatively large cephaloskeleton. They are provided with strongly bent sclerites (gently curved mouth-hooks) and 22-25 terminal spines arranged in two groups. This enables this larva to be separated from that of the first instars of *R.purpurcus* which causes temporary myiasis especially ocular myiasis in humans. The third segment contains a row of denticles on the dorsal side. Ventrally the segments at their anterior margins show two to three rows of spine and hair like structures. Laterally, they have 22-25 hooks Zumpt, (1965).

2.3.3.3. Second stage larvae:

The second instar larva is white in colour and 3.5-12mm long with few weak denticles on the dorsal side of the second segment, the median part of the post –anal bulge is spinulose, ventrally the segments are provided with spines, the posterior peritremes are more or less circular, the channels are indicated by distinct suture Zumpt, (1965). .

2.3.3.4. Third stage larvae:-

The third instar larvae are very similar to one another and are separable only by a feature showing a certain variability ,the third stage larvae is 20-25mm long ,white or yellowish in colour when it is young but changes with light to brown, then showing broad transverse blackish band dorsally after maturity .The second segment is provided with a number of small denticles and the following segment with a rough leather- like skin pattern ,the segment shows strong transverse rows of spines ventrally which are irregularly placed on the third segment , the post anal bulge shows less spines, the posterior peritremes are circular and without suture . The posterior spiracles are exposed, flat, D-shaped plates with the button enclosed with numerous small openings Zumpt, (1965). Plate (2).

2.3.3.5. Puparium:

The pupa is 15-16mm, black, weakly wrinkled.

2.3.4. The Life cycle of Sheep Nasal botfly:

The life cycle of *Oestrus ovis* is divided in two main phases (parasitic phase and free phase), Plate (3).

One day after pupation takes place, the male fly mates the female which hides into the cracks and worm places until summer or spring when it becomes active and attacks the sheep in winter in the tropics (Atafy 1996). The female matures 500 eggs which are deposited as eggs or newly hatched larvae in small batches of less than 50 at a time. When an opportunity presents itself the fly deposits eggs or larvae on or near the nostrils of the sheep or sometimes goats. They may stay there for a month before moving into the frontal and sometimes the maxillary sinuses where they complete their development .The mature larva moves forwards and is sneezed out by the host. It burrows into the soil and pupates. Although nasal bots are in the head, it is not considered as a serious condition, but except for the irritation they cause or the irritation due to the presence of the larvae in the nasal passages interferes significantly with the sheep performance Marsh, (1965).

The first stage larva (LI) is about 1-5 mm in length, feeds on the mucous for about 2 weeks to 9months to develop to LII (15mm) and migrates through nasal passages to the sinuses where it develops to LIII which grows to a length of about 25 mm and a width of 6mm .Mature bots are sneezed and are dropped to the ground where they pupate and the mature fly emerges after 4-6 weeks. The minimum time between the deposition of eggs and the expulsion of mature larvae is 25 days and the maximum is 9months Marsh, (1965).

In the areas where the temperature does not drop below freezing in the winter the fly may be active all the year round but in regions where the winters

are cold the flies appear only in the summer months because the first instars larvae remain quiescent on the nasal mucosa in cold conditions.

2.3.5. Clinical Importance of *Oestrus ovis* larvae:

Small or moderate numbers of bots are usually well tolerated and the infestation is regarded as relatively benign (Rich,1965),cited by Kettle (2000), but annoyance by the adult fly causes sheep to lose valuable grazing time. Sheep flocks show hysterical efforts to prevent the deposition of larvae in their nostrils, stamping their fleets, shaking their heads ,bunching together, holding their noses to the ground or against each other, Zajac and Conboy, (2006).

Mucopurulent discharge and difficult snoring respiration results from irritation caused by the presence of the larvae in the nasal mucosa. Plate (4).

Interference with feeding and rumination reduces weight gain and may reduce milk and wool production. Sneezing and coughing due to the presence of considerable numbers of LI in the nasal passages causes irritation, then secretion of excessive amounts of mucus .Although there are several LIII in the frontal sinuses, these seldom cause any serious inflammation. Occasionally, however, dead larvae are associated with purulent sinusitis.

2.3.6. The Biology of the Oestrus ovis Fly:

Although the nasal bot fly is known as a parasite of nasal cavities and frontal sinuses, some times maxillary sinuses of domestic sheep and goats, other wild animals have been found to act as hosts of this fly e.g. Argali Ibex in central Asia, and a number of antelopes in southern Africa, but all reports are based on miss identification (Bedford, 1925), cited by Zumpt, (1965). So far no antelope or any other wild animals have been found to serve as a suitable host for nasal bot fly in Africa south of the Sahara. (Cobbett and Mitchell, 1941) proved that the life cycle of the fly is greatly affected by the climatic factors, cited by Zumpt (1965).

Development of the first instar larva takes one to nine months .This plays a role in the over wintering cycle and pupal stage lasts 1-2 months depending upon temperature.

Bukshtynov (1978) recoded steady increase in the rate of development of the first instar from 12.5^{o}c to 35^{o}c with adult emergence occurring after 14 day at the highest temperature, cited by Kettle,(2000) .But a temperature above 32^{o}C was fatal, (Rogers and Knapp1973), cited by Kettle (2000). Breev, *et al*. (1980) found that there was increased pupal mortality at a constant temperature of 34^{o}C but the fluctuating daily temperature of 21^{o}c-38^{o}C did not cause mortality, cited by (Kettle, 2000).

Breeding of *Oestrus ovis* would be possible in an area with worm winters all the year-round ,but in many sheep areas where there is a definite cool season ,two generations per annum are considered to occur, however three generations are possible where the flies are active through out the year, (Urquhart *et al* 1996) . In Pretoria there is no larviposition by *Oestrus ovis* in the three winter months of July –September (Horak 1977).

In the northern hemisphere the adult emerges in late spring in June, mates and deposits larvae .The larvae develop rapidly and mature to 3rd instars leaving the host in July and August then pupate to produce an autumn generation .These generations deposit the larvae in September and October .These larvae may remain as first instars for a long period, then become mature in March leaving the host to pupate and remain dormant until June.

The mortality among the immature stages is about 90-94% in first generation and 99% in the second generation (Rogers and Knapp, 1973) cited by Kettle (2000).

In moderate winter the flies become active during most months of the year except January and February. The larval development continues in the winter then become expelled by the host all the year round.

In the cold winter the development of the larvae occurs inside the sheep head ,but the larval infestation is made only by first instar which remain quiescent in the nasal mucosa until the following spring and summer .The flies become active only during the warm days of the summer and early fall.

Cobbet, *et al* (1941) observed grater difference in the larval development in lambs 25-35days in summer but sometimes reach up to 10-11months .Usually the third instar larva needs about 24 hours to pupate ,but it needs 27-28days during worm days of summer .At low temperature it needs 49-66 days (Bedford 1925) cited by Zumpt,(1965).

The longevity of the flies in captivity varies and depends on the temperature. Cobbett and Mitchell (1941) were able to keep the flies for half a day in the worm days of summer, cited by Zumpt, (1965). But Fallis (1940) in Canada gives average of survival times of 16 days cited by Zumpt, (1965).The female flies normally live up 25 days but some had been kept alive for up to 68 days. The dissection of female flies revealed an average number of 500eggs. The larvae are deposited in batches of one to several dozens.

In South Africa, separate groups of 3 Oestrid –free lambs were exposed to infestation on irrigated pasture for a period of approximately 33days each over 30 months and on dry land pasture for approximately 42 days over a period of 18months. With some exception, the lamb slaughtered from October to June was found to be infested with *Oestrus ovis*, while with one exception, those slaughtered from July –September were free. Horak,(1977).

2.3.7. Pathogenesis:

As mentioned earlier the larvae and pupae are regarded as relatively benign and are responsible for comparatively mild effects ,but the continuous irritation produced by the cuticuler spine ,oral hooks of the larvae and toxic substances excreted by the larvae profoundly affects the well being of the infected animals .

The degree of pathogenicity is greatly affected by many factors e.g.

-Different resistances of the various breeds of sheep and goats.

-Number and location of first and second instars larvae in the individual animal .Plate (5).

Environmental factors should be taken into consideration like the state of nourishment and the climatic conditions

Grunim (1957) reported that more than 350 larvae of all stages have been found in one sheep's head during a dry year. They are responsible for high mortality specially in lambs and specially when they penetrate the bronchi. The larvae may erode the bones of the skull and enter the brain leading to incoordination and other symptoms similar to *Coenurus cerebralis* infection (Geoffrey and lapage 1968).

The incidence in infected sheep and goats varies considerably with different seasons in different areas.

When the pregnant flies do not find the suitable host quickly enough to deposit their eggs they attack man and dogs causing ocular myiasis (ophthalmomyiasis), however in unsuitable hosts the larvae do not develop beyond the first stage. Man is affected in areas where the number of sheep and goats is relatively low. Humans become exposed when they are handling these animals and are contaminated with their odour. In parts of northern Africa it is claimed that man is infected when he eats goat cheese .But this condition need not necessarily exist, and quite often people who have had nothing to do with animals at all are attacked, Zumpt,(1965).

In man the fly does not drop its eggs into nostrils, mouth or outer ears but into the orbit and the patient reports being struck by an insect or foreign object in the eye. Painful inflammation develops and the condition is diagnosed as catarrhal conjunctivitis which lasts only a few days but some times the larvae reach the nasal cavity causing painful swelling and frontal headache takes place. It may cause inflammation of the throat and difficulty in swallowing .These symptoms disappear gradually, and rarely take more than 10days.80 such cases

were treated over 2 years at a clinic in Benghazi, (Dar *et al* 1980), cited by Kettle (2000) Also they reported three cases of *Oestrus ovis* infestation in Italy in a limited area of laspezia province during summer 2004, none of the patients had contact with wild or farm animals. *Oestrus ovis* is also responsible for causing Stomatitis and gingivitis in humans, (Elkan 2004)

The larvae due to their small size and transparency are difficult to be seen and the cause of conjunctivitis maybe missed. As many as 50 larvae have been found in conjunctival sac of one patient and the course of the disease is always benign .The larvae do not invade the eye ball .The infection is known as(Thimi) in Algeria and as(Tamnein) in Alhaggar mountains of central Sahara .

2.3.8. Distribution of *Oestrus ovis*:

The spread of *Oestrus ovis* has been promoted by the importation of large numbers of infested sheep. However its survival in new habitats is due to the wide range of its temperature requirements. The humidity plays relatively minor role in its survival (Rogers and Knapp 1973), cited by Kettle (2000).

Also they reported three cases of *Oestrus ovis* infestation in Italy in a limited area of Laspezia province during summer 2004; none of the patients had contact with wild or farm animals. *Oestrus ovis* is also responsible for causing Stomatitis and gingivitis in humans (Elkan 2004).

A case of oral mucosa myiasis caused by *Oestrus ovis* in a three –year old boy from a shepherd family living in rural area of Iran is being reported by (Hakimi *et al* 2002) Plate (6) .

To describe and diagnose a human case of ophthalmomyiasis caused by the sheep botfly *Oestrus ovis* larvae, the authors emphasized that the importance of collaboration between practitioner or specialist and prasitologist for a sound, definitive an etiological diagnosis of such uncommon pathologies is very important (Crotti 2005).

2.3.9. Laboratory Diagnosis of Sheep Nasal Bots:

The larvae are not usually accessible for identification because of their location, but they are some times found by owners in water troughs or on the ground. Specific identification is made by examination of the spiracular plates, Zajac, (2006).

2.3.10. Control:

Control of nasal bot is comprised of two parts:

A. Vector control: It is aimed to rill the adult flies before they can cause any damage i.e. prevention. Wikipedia org. (2008)

B. Treatment once the infestation takes place in the infected animals or humans.

2.3.10.1. Prevention Methods:

1- Application of insecticide in the environment of the target livestock where they are kept using organophosphorus or organochlorine compounds usually in a spray formulation.

2- SIT (sterile insect technique), (http: //en. Wikipedia. org. /wiki /Myiasis (2008) .Significant numbers of artificially reared sterilized male flies were introduced in the field to compete with wild males for females in order to copulate and cause the females to lay batches of unfertilized eggs which can not develop into larval stages. .

3- Application of wood tar to the nostrils of sheep. It acts as insects repellent. Application must be repeated weekly .Repellent applied to the muzzles of the sheep are usually less effective because their effect does not last long on the hot sunny days when the flies are active.(Geoffrey and Lapage 1968).

4- Because the life cycle of the fly is relatively short , continuous moving from grazing area to another is a very important way of prevention, (Elbaroni 1979) .

2.3.10.2. Treatment:

Its may not be economically worthwhile to treat moderate infestation, however in heavy infestation treatment is very important in warm climates where the fly and its larvae interfere significantly with sheep production. The methods of treatment are:

1-Using slow release boluses of ivermectin to provide long term protection against larval development.

2-Drenching: - use of organophosphorus compounds orally e.g.

A. Dowco (109 grams) kills all stages at 75 mg /kg (Peterson *et al*.1960) cited by Marsh, (1965).
B. Bayer 37342 kills all stages at 50 mg /kg (Drummond 1961) cited by March, (1965).
C. Other slightly less effective compounds are Ruelene, Neguvon, and dimethoate in 4 annual treatments orally. The average number of larvae per sheep drops from 50 in 1958 to 8.7 in 1962, (marsh, 1965).

3- Injection of one ounce of 3% saponified cresol(Lysol) into each nostril with sheep held on its back with head down flat (Cobbit,1940a 1940b,1956),cited by Marsh(1965) ,this treatment is applied in late fall after the fly season is passed, it eliminates more than 90 % of the first stage larvae causing .Newsomes sheep disease (Marsh. 1965).

4- Using a mixture of 12.5 parts by volume of benzol, 12.5 parts acetone,
10 parts kerosene, 57 parts sulphonated caster oil, 2parts Tricon *100 six parts oleic acid and 4g /100ml lindane, the dose is 4 ml / nostril /adult sheep. Lamb and lactating ewe should not be treated .

A single injection of each nostril clears the larvae from individual sheep, due to reinfestation at three treatments every 3-4 weeks is recommended. Du Toit and Fiedler Newsoris Sheep Disease (Marsh 1965).

5- Rafoxinide, nitroxynil, trichlorfon and dichloride are highly effective.

Treatment should be given twice a year, the first at the beginning of

summer to kill newly-acquired larvae , and the second treatment at mid winter to kill any over wintering larvae (the scheme suggested by South African workers),(Urquhart *et al* 1996).

6-spray of ether extracts of male fern into the nostrils. (Gan, 1954) in the U.S.S. R. cited by Zumpt, (1965).

7- Application of Trichlorphon at the rate of 52-70 mg /kg body weight to the nostrils is with effective result, and is a recommended treatment at intervals of 4weeks,(Slampa and Polls 1960), South Africa, cited by Marsh (1965).

Treatment in October –November was suitable because first instars were in diapauses preventing the development of first instars into the second instars, and second instars into third instars. (Scala 2002).

During two years trials were conducted on 100 Merino lambs showing signs of *Oestrus ovis* infection to determine the efficacy of rafoxanide.The treated group was virtually free of this parasite and showed reduction of nasal discharge and an increased weight gain. A suggested program for strategic drenching of sheep in Transvaal Highveld was out lined (Horak and. Snijders. 1974.).

2.3.11. Collection and Rearing of *Oestrus ovis* Adults and Larvae:

The naturally dropped mature maggots of sheep can be obtained by keeping the animals in stables with wooden crate on the floor. The larvae will crawl through the gaps and pupate in the soil beneath the crate so they can be collected. Also larvae can be collected from opened skulls, some will pupate and yield adults if isolated in sand and some will die .

Collected larvae (all stages) may be dropped into 70 % Alcohol at once, and labeled. If possible it is better to kill them in hot water before they are dropped into the preservation fluid, because the larvae become stretched and show their morphological features better.

Hatched flies should be treated with special care. They usually remain crippled for unknown reasons and don't stretch their wings. Fully developed flies should never be dropped into a fluid but killed and preserved under dry condition using (chloroform, ether or ethyl acetate vapour and then mounted on a pin, but pinning of flies should be done by experienced persons (Zumpt 1965) .

CHAPTER THREE
MATERIALS AND METHODS

3.1. Area description:

The study was conducted in Alsalam Abattoir which is located on an area of 1500 square meter west of Omdurman city in Alsalam Administrative unit, Ombadda Locality, Khartoum State.

Although Alsalam abattoir is not a standard slaughter house and it is regarded as third degree slaughter house but there are large numbers of daily slaughtered animals specially sheep during two periods of the day. The animals are collected from nearest three livestock markets (Alsalam live stock market, Gendhar livestock market and Elmowelih livestock market), which receive those animal from different sources and sites of the country.

3.2. Materials:

3.2.1. Sampling:

The study was conducted during the months March and May 2009. With the exception of 6 days, 7 to 20 sheep's heads were collected from the abattoir each day during March and ten sheep heads were collected from the same abattoir each day during May using convenience sampling technique. The heads were examined on dissection table for *Oestrus ovis* infestation using different dissection tools. Larvae collected from each head were placed in different small bottle containing 70% ethyl alcohol for identification and measurement.

3.2.2. Preparation of materials:

Dissection microscope.

Petri dishes

Filter paper

Dissection tools

Methods:

3.3.1 Fly rearing:

Using Forceps mature third instars larvae were placed in large glass bottles containing sand, 5-6 larvae in each bottle covered with gauze which is fixed by flexible band. Five bottles were incubated at room temperature and other ten bottles incubated at 28^{o}C using an incubator (Thermostatic cabinet), plate (7).

After pupation takes place the emerged flies were grouped in two batches. Batch (1) placed in bottles containing 70% alcohol and glycerin. Batch (2) placed in test tubes and covered with cotton for identification.

3.3.2. Identification:

Identification of different stages of larvae and emerged adult flies was conducted using Zumpt Key with the aid of dissection microscope in the Parasitology Laboratory, Faculty of Veterinary Medicine.

The percentage of each larval stage collected, was calculated. Also the mean larval burden and percentage of each larval stage in each sheep's head, was assessed.

CHAPTER FOUR

RESULTS

Of the 567 heads of sheep collected, 98 heads were found to be infested with *Oestrus ovis* larvae. The infestation rate of nasalbot fly in Sheep heads was therefore 17.3%.

The total number of larvae collected during the study from the 98 heads was 151.Identification of these larvae showed the following results: -

136 larvae were found to be third instars, 15 larvae were second instars, while no first in star larvae were found .Plate (8) shows these different larval stages, Plate(9) and Plate (10) show the *Oestrus ovis* third instar, dorsal and ventral view respectively.

The mean larval burden in sheep heads examined during the study was found to be 1.5, while 13 larvae of different stages was the maximum number of larvae recovered from one sheep head.

The numbers ranged between 1-----13 larvae.

The percentage of each larval stage in the total of collected larvae was found to be 9.9% for the second instars where as third instars constitute the largest part of the larvae (90.1 %).

Identification of larvae collected from each sheep head during the study was ranged between 0 % -100% for both second and third instars.

The pupal period of the larvae which were allowed to pupate and hatch at room temperature during the worm days of March and May ($38^{o}C$) was 15-21 days but for larvae which were incubated in thermostatic cabinet at $28^{o}C$, it was between 17-23 days.

Table (1): The numbers and percentages of second and third instars considering the total collected larvae.

Heads No.	No. of second instars	No. (%)	No .of third instars	No. (%)	Total
98	15 Larvae	9.9%	136 Larvae	90.1%	151Larvae

When 32 larvae were allowed to pupate and hatched at room temperature during March and May (38^{o}C), 21 flies emerged and the pupation percentage was found to be 65.6% as illustrated in but when 54 larvae pupated and hatched in thermostatic cabinet at 28^{o}C, 31 flies were emerged with pupation percentage of 57.4 % as shown by figure. The total numbers of flies that emerged after incubation tock place during the study were 52 flies. All emerged flies were similar. They showed the same colour and the same morphological features when examined under the dissection microscope. These morphological features specially the black pits in the abdomen and wing venation revealed that all emerged flies were *Oestrus ovis* species. Pate (11) show the emerged adult *Oestrus ovis* fly.

Flies that emerged in the first day of November remained viable for more than eleven days when they are kept in covered Petri dishes.

Table (2): Emergence percentages in different rearing environments.

Rearing Environment	**No. Incubated**	**No. emerged**	**Emergence %**
Room Temp. (35^{o}c)****	32 larvae	21 Flies	65.6%
Incubator (28^{o}c)***	54 larvae	31 Flies	57.4 %

CHAPTER FIVE
DISCUSSION AND CONCLUTION

5.1. Discussion.

As different stages of *Oestrus ovis* larvae were detected in the nostrils of sheep, this indicates that sheep nasal bots infestation could take place regardless of season. Some opened sheep skulls were found to be free of infestation. This observation indicates that sheep may have been gathered from different sites of sheep production and may exhibit different levels of immunity, or they could have suffered from infection only at random.

In the present study all emerged flies were Oestrus *ovis* judging from their morphological features. This observation showed that no other flies than *Oestrus ovis* had been responsible for nasal myiasis in sheep, according to many reviews of literature over the past 84 years from (1925—2009) e.g. Bedford,(1925) , Cobbett and Mitchell(1941),cited by Zumpt,(1965).And Horak,(1977).

In contrast the cutaneous myiasis may be highly fatal unlike nasal myiasis , because in cases of cutaneous myiasis the resultant lesions would be invaded by bacteria leading to toxemia ,anorexia , weakness and death.

In this study the pupation period of incubated larvae ranged between 17 to 23 days for flies that hatch normally. This incubation period is shorter than that reported by (Horak, 1977) in South Africa is (60days); such differences may be attributed to seasonal differences in the two localities.

In this study the emerged flies were grey green in colour, this finding agreed with what is reported by Hall, (1977), but they appeared yellow brown in colour under the dissection microscope as reported by Zumpt et *al* (1965).

In this study flies emerged in the first day of November (during winter) remained viable for more than 11days. Horak (1977) reported an average lifespan of emerging flies as one and half days during summer. Cobbet and Mitchell, (1941) reported life span of 8 days during autumn; where as Fallis (1940) in

Canada reported an average of 16 days. This reveals that adult fly's life span is found to be longer during moderate winter and shorter during summer. These apparent variations in the survival of the emerged flies indicate that flies do not survive the high temperature of the summer season.

The infestation rate of *Oestrus ovis* in sheep heads examined during this study was found to be 17.3% which is less than that reported by Gabaj *et al* (1992) in northern Libyan sheep and goats. This variation may be attributed to the locality and seasonal differences and the severity of the fly challenge in both Sudan and Libya.

In this study the maximum numbers of the larvae collected from only one sheep head was 13 larvae, but Grumin (1957) where was able to collect more than 350 larvae from an individual sheep head. An observation showed 11and14 larvae were collected from individual sheep and goat respectively Gabaj, (1992) .These different findings in the degree of infestation may be due to many factors like general health of the animals, nutritional status , climatic conditions and the number of adult flies around them.

In the present study no first instars larvae were seen when the skulls of sheep were opened , this finding agreed with Gabaj *et al* (1992) as first instars larvae normally recovered from the nasal passages and frontal sinuses , are transparent and small in size .

The mean larval burden in sheep heads examined during the study was 1.5 larvae, which is less than that reported by Horak (1977) in South Africa reported 15.2 larvae in sheep and 4.4 larvae in the goats, but Horak *et al* (1977) reported mean larval burden of 15 - 22 larvae per sheep.

Bukshtynov (1978) recorded steady increase in the rate of larval development at temperatures ranging from 12.5C· to 35C cited by Kettle, (2000), whereas marsh (1965) reported different periods of larval development ranging from 25days to 9 months. Cobbet *et al* (1941) recorded 25 to 35 days in summer

but sometimes this reached up to 10- 11 months .These greater differences show the considerable effect of temperature on the rate of larval development.

In this study the percentage of pupal mortality at 28^{o}C was greater than the percentage of pupal mortality at 38^{o}C. Breev, *et al* (1980) reported an increase in pupal mortality at a constant temperature of 34^{o}C, cited by Kettle, (2000). They also reported constant mortality rate at fluctuating daily temperatures of 21^{o}C – 38^{o}C.Therefore the finding in this study disagrees with their results.

Horak and But (1977) reported pupation percentage of 52.8% in Johannesburg Municipal Abattoir which is less than 65.6 % when the larvae were allowed to pupate and hatch normally at room temperature. This is because Horak (1977) conducted the study over a longer period than the period of this study of only two months.

There is no considerable difference between the length of two pupation periods during this study but the differences which were clearly reported by different authors in different localities at different times were as short as 14 days and as long as 66days, is almost due to the variation in climatic conditions and other unknown factors.

Horak (1977) reported 73.8% mean incidence rate of *Oestrus ovis* in goats in South Africa but Unsworth (1949) in Nigeria reported 15% incidence rate in goats in March and 64% in May .

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5.2. CONCLUSION AND RECOMMENDATION:

1. More research and further investigation in nasal bot fly myiasis is important with the objective to determine its economic effects on target animals and as a zoonosis.
2. In addition particular attention must be directed towards an accurate assessment of mortality and production losses due sub clinical cases of sheep nasal botflies as well as morbidity and mortality in preslaughter ages. Therefore more studies are needed for planning for a control program for the disease.

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APPENDICES

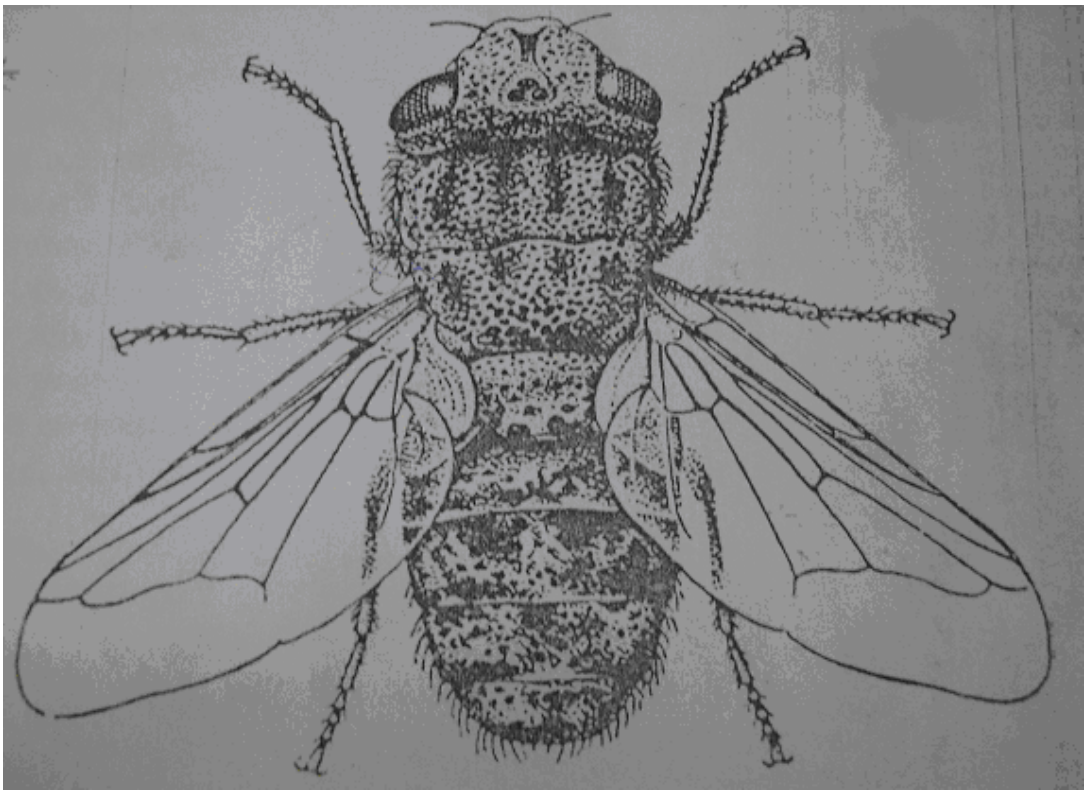


Plate: (1): *Oestrus ovis*. Female Fly.

Source: Myiasis in Man and Animals in the Old World, (Zumpt, 1965)

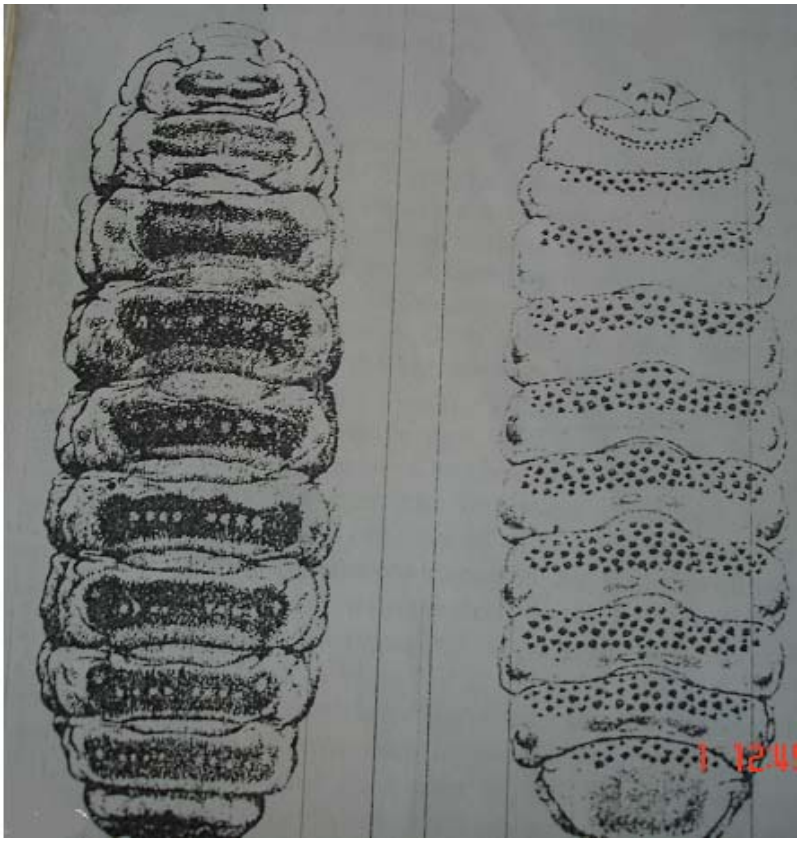


Plate (2): *Oestrus ovis* larvae. Dorsal and ventral view (Zumpt, 1965)

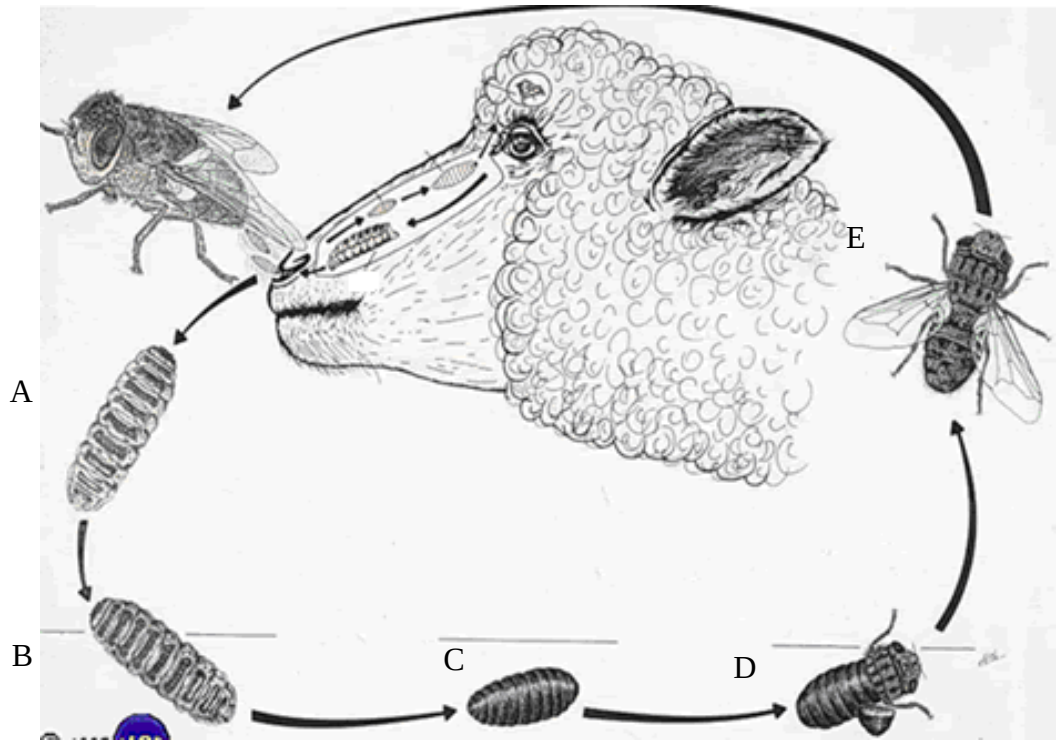


Plate (3): *Oestrus ovis* life cycle

A: Immature 3rd instar larva.

B: Mature 3rd instar larva.

C: Pupa in the ground.

D: Adult fly emerges from the pupa.

E: Mature fly.

Source: Marcello Rojasc (2008).

Plate (4): *Oestrus ovis* infected sheep. difficult breathing

Note open mouth and congested nostrils.

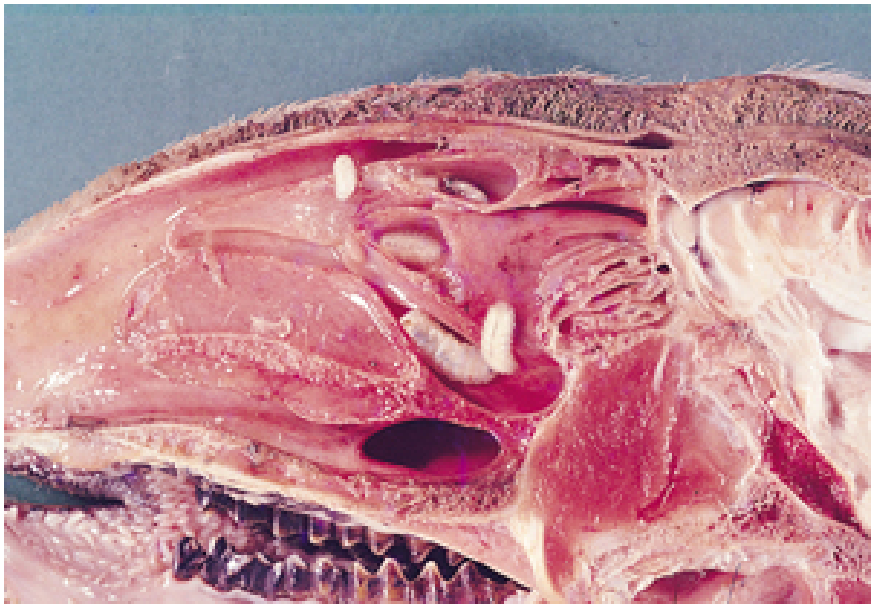


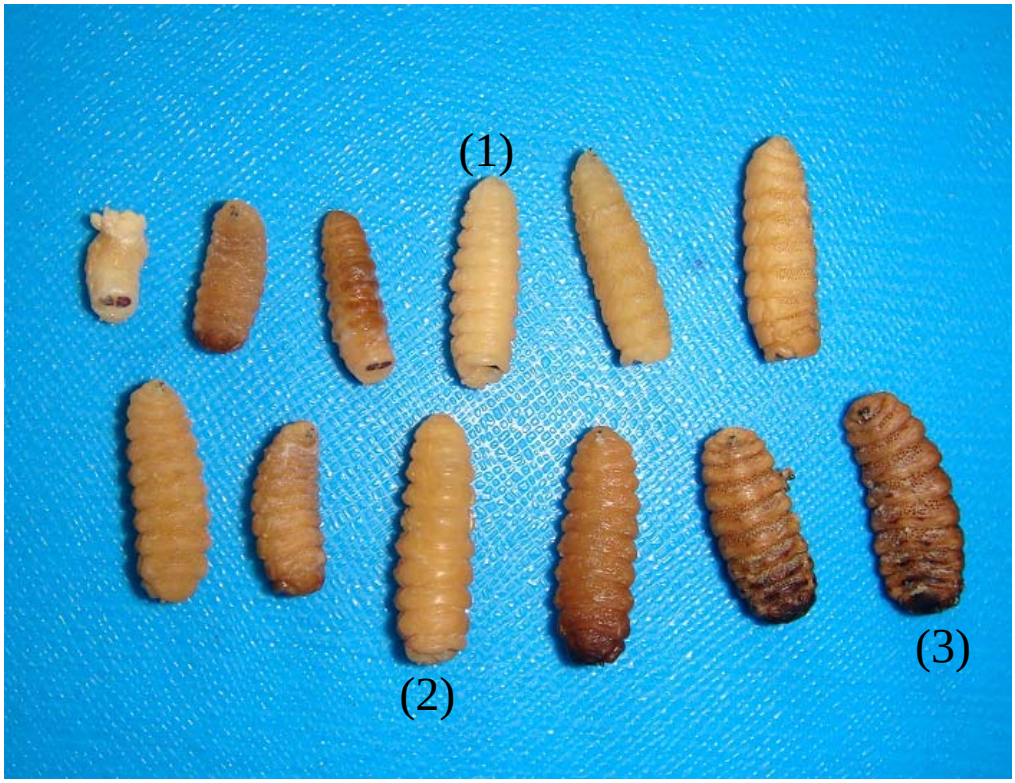
Plate (5): *Oestrus ovis* larval predilection. Skull section of sheep

Source: Caparaispana com/noticia/2007/marzo

Plate (6): *Oestrus ovis* Human oral larval infection (myiasis)

Source: R Hakimi and I Yazdi (2002).Case Report.

Plate(7) :Incubation (rearing of *Oestrus ovis* larvae / pupae)



Pate: (8) Different Larval Stages.

1- **Second instar.**
2- **Immature third instar.**
3- **Mature third instar.**

Plate: (9) *Oestrus ovis* larva -dorsal view.

Plate (10): *Oestrus ovis* larva ventral view.

Plate (11): An adult fly emerged from an incubated larva / pupa.